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Skin colonization by *Malassezia* species in healthy neonatal calves and their dams

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Abstract

The aim of this study was to assess skin colonization by *Malassezia* species in full-term healthy newborn calves and their dams. Two hundred samples from the skins and ears of 50 neonatal calves and their dams were examined on two occasions, first and fourth weeks after birth**.** All of the samples were determined by cytological examination and fungal culture. The isolated yeasts were identified for *Malassezia* spp. using the conventional techniques based on the morphological and physiological characteristics. All the samples included in the first and fourth weeks showed typical *Malassezia* cells on cytological examination**.** Colonization with *Malassezia* species was obtained in 11.5% of neonatal calves and their dams. The most commonly isolated species in neonates with culture-positive results was *M. pachydermatis* (68.75%), followed by *M. sympodialis* (12.5%), *M. furfur* (6.25%), *M. globosa* (6.25%) and *M. slooffiae* (6.25%).The most commonly isolated species in dams with culture-positive results was *M. pachydermatis* (85.71%), followed by *M. furfur* (14.3%). This study confirms that *Malassezia* colonization of the skin begins at the first week of life. A high prevalence of *M. pachydermatis* in neonates is noted from first week. Environmental factors and maternal contact probably affect this colonization, but neonatal skin characteristics are probably important.

Keywords: neonatal calves**,** dams, skin colonization, *Malassezia* species , *M. pachydermatis***.**

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Introduction

The genus *Malassezia* forms part of the physiological skin flora but is also implicated in the pathogenesis of common skin diseases such as pityriasis versicolor, atopic dermatitis, seborrheic dermatitis and psoriasis. Additionally, the species *M. pachydermatis* and *M. furfur* can cause systemic infections and small epidemics in human neonatal and immunocompromised adult intensive care units (Gaitanis *et al.,* 2009). It has been widely speculated that the source of the strains causing fungemia in children is the skin of parents or healthcare workers, but it is not possible to test this hypothesis because of the lack of epidemiological typing methods for *Malassezia*. Although it can be implicated in some systemic neonatal infections (Bell *et al.,* 1988), *Malassezia* species in healthy neonates are associated with the common acne-like pustulosis of the cephalic area (Bernier *et al.,* 2002; Rapelanoro *et al.,* 1996). Newborn skin is sterile at birth, but resident flora may be detected within the first hours of life. Both the age at which neonates become infected and the route by which healthy neonate skin is colonized with *Malassezia* is a matter of controversy (Ayhan *et al.,* 2007). The studies about this subject have documented results that differed considerably, ranging from no colonization by *Malassezia* to substantial colonization (Ahtonen *et al.,* 1990; Bernier *et al.,* 2002; Leeming *et al.,* 1995; Shattuck *et al.,* 1996). Recent identification and differentiation of *Malassezia* species have opened new avenues for investigations. There is no data on skin colonization by *Malassezia* species in animal neonates. To the knowledge of authors this is the first study on colonization by *Malassezia* species in neonatal calves and their dams. The aim of this study was to assess skin colonization by *Malassezia* species in full-term healthy newborn calves and their dams.

Materials and methods

Animals

Between August 2011 and February 2012, two hundred samples from the skins and ears of 50 neonatal calves (21 males and 29 females) and their dams were examined in the Mycology Laboratory of the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran. Each animal was sampled on two occasions, first and fourth weeks after birth. The samples were obtained from two commercial dairy herds located in Mashhad (northeast of Iran, latitude, 36°20'N; longitude, 59°35'E E; altitude, 985 m), Iran. The herds consisted of Holestein-Frisian dairy cows and had an average of 750 and 860 lactating dairy cows in autumn 2011. The area usually experiences a relatively hot summer extending from May to October with almost no rainfall. Age, sex, birth weight of calves, demographic characteristics, antimicrobial drug use, gestational age and the other clinical data for newborn calves and their dams were recorded.

Procedures and laboratory methods

All samples were taken by a single person from the same area of skin and one ear of each animal in an attempt to standardize the procedure. Sampling was performed from each animal by using six sterile cotton swabs(2 swabs for cytology and 4 swabs for culture) moistened with sterile saline solution (0.9% NaCl) on a 4-cm^2 area of junction of the neck to the shoulder and the external canal of left ear.

The initial analysis for detection of *Malassezia* species was performed by direct microscopy examination of a smear after May-Grünwald-Giemsa staining. For isolation and identification of *Malassezia* species, skin and ear swabs were seeded onto the surface of Sabouraud dextrose agar (SDA) and modified Dixon agar. The plates were incubated at 32 °C for 7-10 days. *M. pachydermatis* was identified by morphology microscopically and by the ability to grow on SDA. When growth was detected on solid media, a maximum of five different colonies were selected from modified Dixon agar for identification of the lipid-dependent yeasts.It was based on the

ability to use certain polyoxyethylene sorbitan esters (Tweens 20, 40, 60 and 80), as described by Gueho *et al.* (1996), and catalase reaction proposed by Guillot *et al.* (1996). The Cremophor EL assimilation test, the splitting of esculin described by Mayser *et al.* (1997) and growth on Dixon medium at 40°C reported by Gueho *et al.* (1996) were used as additional tests.

Statistical analysis

All statistical analyses were performed using SPSS statistical software (SPSS Inc, Chicago, Ill; and Stat Exact, Version 8.2, Cytel Inc, Cambridge, Mass). Data obtained from the present study were analyzed by *chi-square* test. A *P*- value less than 0.05 was considered significant.

Results

All the newborn calves remained healthy and neither the neonates nor the dams had received oral / injection antibiotics or antimicrobial local treatment during the study period. The age of the 50 dams ranged from 2 to 11 years (median 4.12 yrs). Of the total neonatal calves studied, 21 were male and 29 were female.

There was no statistical effect of sex and gestational age on *Malassezia* colonization. The only factor associated with colonization was the birth weight of calves. The average of birth body weight of negative calves (44.24± 5.75 kg) significantly was higher than positive calves (40.23± 4.27 kg) (P= 0.03).

*Microscopic examination***:** All the samples included in the first and fourth weeks showed typical *Malassezia* cells on microscopic cytological examination.

*Fungal culture***:** Colonization with *Malassezia* species was obtained in 23 out of the 200 (11.5%) neonatal calves and their dams. In the neonatal population studied in both weeks, 16 samples out of 100 samples (14 in first week and 2 in fourth week) became colonized with *Malassezia* organisms; so that 10 of whom were isolated from skin and 6 from ear. Seven samples out of 100 samples (5 in first week and 2 in fourth week) of 50 dams had positive culture results for *Malassezia*; so that 4 of whom were isolated from skin and 3 from ear.

In examined neonates, there was significant difference between the frequency of isolation of *Malassezia* yeast from skin and ear in first week compared with fourth week ($p < 0.05$). The frequency of isolation of *Malassezia* yeast from calves in first week was significantly higher than that of the fourth week ($p = 0.003$).

In dams, there was no significant difference between the frequency of isolation of *Malassezia* yeast from skin and ear in first week compared with fourth week after parturition.

Totally, the frequency of isolation of *Malassezia* yeast from all animals studied in first week was significantly higher than that of the fourth week ($p = 0.002$).

A total number of 23 strains from five *Malassezia* species isolated from newborns and dams were detected with a frequency rate of: *M. pachydermatis* 17 (73.91%), *M. sympodialis* 2 (8.69%), *M. furfur* 2 (8.69%), *M. globosa* 1 (4.34%), *M. slooffiae* 1 (4.34%).

The most commonly isolated species in neonates with culture-positive results was *M. pachydermatis* (68.75%), followed by *M. sympodialis* (12.5%), *M. furfur* (6.25%), *M. globosa* (6.25%) and *M. slooffiae* (6.25%).

The most commonly isolated species in dams with culture-positive results was *M. pachydermatis* (85.71%), followed by *M. furfur* (14.3%).

There was no relationship between calves and their own dams. Regarding isolated *Malassezia* and different species of is isolated *Malassezia*.

In doing so, in addition to *Malassezia*,we were able to isolate other fungi such as *Candida*, *Trichosporon* and *Geotrichum* in 17% of all samples; so that the most common fungal isolate was *Candida* spp.(70.5%), followed by *Trichosporon* (23.5%) and *Geotrichum* (6%).

Discussion

Yeast of the genus *Malassezia* is one of the normal microbiota elements of adult skin. The colonization rates of this yeast are variable according to age and site of skin sample. In spite of our knowledge of colonization in adults, colonization of this yeast in the neonate population is needed to be more clarified. There are variable reported results, ranging from no colonization to substantial colonization of this micro-organism in neonate skin. Previous works demonstrated that the ears, trunk and forehead are most likely to be colonized with *Malassezia* in both adults (Leeming *et al.*, 1989) and neonates (Ashbee *et al.*, 2002; Leeming *et al.*, 1995). Thus, samples were collected from ear and trunk in both neonatal calves and dams in the present study,

In both groups of studied animals, microscopic examination of roll smear cytology showed that all samples were positive; whereas, a large number of samples with positive direct microscopy failed to yield a culture. The cytological examination results were considered positive even if only one yeast cell of *Malassezia* species, from trunk or ear canal, was observed in 5 random fields at 40 × magnifications; while, small numbers of *Malassezia* cells in samples is extremely difficult to propagate in laboratory culture. Furthermore, concomitant carriage of *Candida* and the other yeast flora associated with the skin inhibit the growth of *Malassezia*.

The prevalence of *Malassezia* species in human neonates has been reported to be 13% and 50% in the first week of life by different authors (Ahtonen *et al.*, 1990; Ashbee *et al.*, 2002; Bell *et al.*, 1988; Koseki and Takahashi 1988; Powell *et al.*, 1987; Shattuck *et al.*, 1996). These studies were performed in intensive care departments, with premature infants or systemically ill neonates, and in a wide range of age groups. In the current study, the frequency of isolation of *Malassezia* yeast from neonatal calves in the first week was significantly higher than that of the fourth week; so that the colonization on the first week of life is 14% and decreased to 2% after four week. In contrast, Bernier *et al.* (2002) reported that the colonization rate in the first 5 days of life, in 102 healthy human neonates, was found to be 11% and at day 21 of life, it was 52%. Also, Ayhan *et al.* (2007) showed that the colonization rate in 104 human neonates with and without cephalic pustulosis on the first 3 days of life was 5% and increased up to 30% after the first week. These differences might be the results of the sampling time/site, number of the neonate studied, direct contact with mother/nursery personnel/other family members, stay in an intensive care department and ill neonates. On the other hand, in the present study, increased colonization in the first days of life in neonatal calves could be due to the transient flora originating mostly from the dam’s genital tract (Leyden 1982); whereas, decreased colonization observed in future days is because of neonate's intrinsic characteristics especially sebum production in pre puberty. Sebum is produced under hormonal control, with sebaceous gland active at birth under the control of maternal androgens. They quickly reduce in size and sebum production until the onset of puberty. As puberty begins the sebaceous gland again become activate, this time is under the control of circulating androgens (Ro and Dawson 2005).

In the dams, the prevalence of *Malassezia* species is 10.5%. This rate is lower than several previous studies that had reported that the *Malassezia* species are detected in significant frequencies, ranging from 16% to 33% in ear and skin samples of healthy cattle (Duarte and Hamdan 2008; Duarte *et al.*, 1999). This difference can be explained by the use of Leeming and Notman agar, which has been shown to recover *Malassezia* more reliably than other media (Ashbee *et al.*, 2002; Leeming *et al.*, 1989).

The rate of *Malassezia* colonization at the first week was higher in neonates than dams (14% vs 5%).The elevated flow of sebum in neonates owing to sebaceous gland

hyperactivity could partially explain this finding.

Regarding the isolated species, *M. pachydermatis* was most commonly cultured from the skin and ear in both the neonatal calves and their dams. The other species of *Malassezia* isolated from studied animal included *M.* sympodialis, M. *furfur*, *M. globosa* and *M. slooffiae* in neonatal calves and *M. furfur* in dams. Crespo *et al.* (2002) reported that *M. sympodialis*, M. *furfur*, *M. globosa* and *M. restricta* were isolated from skin and ear of cattle in Spain. In Brazil, Duarte *et al.* (1999, 2003, and 2008) noted that *M. sympodialis*, M. *furfur*, *M. globosa* and *M. slooffiae* were recovered from cattle with and without otitis.

In contrast to our study, Crespo *et al.* (2002) and Duarte *et al.* (1999, 2003, and 2008) did not isolated *M. pachydermatis* from their samples. Moreover, contrary to our study, Hirai *et al.* (2004) reported *M. nana* from cattle with and without otitis. These differences could be due to various climatic and breed differences, the sampling technique used, the greater number of skin areas sampled and the used of different media to allow the isolation of lipid-dependent species.

Furthermore, as mentioned in the results part, of 16 *Malassezia*-positive neonates, their dams were *Malassezia* negative. This matter can be due to few numbers of animals studied.

In conclusion, the present work confirms both the presence of *M. pachydermatis* as the most prevalent species in the skin and ear of neonatal calves and dams, and the presence of some lipid- dependent species of *Malassezia*. Also, *Malassezia* colonization of the skin begins at first week of life. A high prevalence of *M. pachydermatis* in neonates is noted from first week. Environmental factors and maternal contact probably affect this colonization, but neonatal skin characteristics are probably important. Further investigation is needed to study the role of sebum secretion rate and quality in the neonatal period. It is suggested to use the greater number of animals and to address the role of culture medium specificity for the cultivation of each species in other studies. Furthermore, it would be interesting to study comparatively the species/genotype of *Malassezia* isolated for each couple calf/dam, and also to sample the dams at different times during pregnancy before parturition.

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کلونیزاسیون پوستی توسط گونه های *مالاسزیا* در گوساله های نوزاد سالم و مادرانشان

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چکیده

هدف از مطالعه حاضر ارزیابی کلونیزاسیون پوستی توسط گونه های *مالاسزیا* در گوساله های نوزاد سالم و مادرانشان بود. ۲۰۰ نمونه از پوست و گوش ۵۰ گوساله نوزاد و مادرانشان در دو مرحله، هفته اول و هفته چهارم بعد از تولد مورد آزمایش قرار گرفتند. همه نمونه ها از لحاظ آزمایش میکروسکوپی مستقیم و کشت قارچی بررسی شدند. به منظور تعیین گونه مخمرهای جداسازی شده از تکنیک های معمولی بر اساس خصوصیات مورفولوژی و فیزیولوژی استفاده گردید. در آزمایش میکروسکوپی مستقیم، تمام نمونه ها، سلول های مخمری تیپیک مالاسزیا را نشان دادند. کلونیزاسیون با گونه های مالاسزیا در ۱۱.۵٪ گوساله های نوزاد و مادرانشان مشاهده شد. معمول ترین گونه جداسازی شده در نوزادان با نتایج کشت- مثبت شامل به ترتیب *مالاسزیا پکی درماتیس*۶۸.۷۵٪، *مالاسزیا سیمپودیالیس*۱۲.۵٪، *مالاسزیا فورفور*، *مالاسزیا گلوبوزا* و *مالاسزیا اسلوفیه* هر کدام ۶.۲۵٪ بود. معمول ترین گونه جداسازی شده در مادران با نتایج کشت- مثبت شامل به ترتیب *مالاسزیا پکی درماتیس* ۸۵.۷۱٪ و *مالاسزیا فورفور* ۱۴.۳٪ بود. این مطالعه تأیید می کند که کلونیزاسیون پوستی *مالاسزیا* در هفته اول زندگی شروع می شود. یک شیوع بالایی از *مالاسزیا پکی درماتیس* در نوزادان از هفته اول بع د از تولد ثبت می شود. احتمالا " فاکتورهای محیطی و تماس مادری این کلونیزاسیون را تحت تاثیر قرار می دهد، اما خصوصیات پوست نوزادان نیز مهم می باشد.

واژگان کلیدی: گوساله های نوزاد، مادران، کلونیزاسیون پوستی، گونه های *مالاسزیا*، *مالاسزیا پکی درماتیس*